of bone explants cultured with estradiol prevented this increase in a dose-dependent manner (102.5±8.2% at 10nM; 73.4±7.7% at 1000nM; p=0.049) as well as with pamidronate (79.6±21.8%; p=0.049). The same results were observed with ARGSV WB with a 41% decrease with estradiol at both doses and 61% decrease with pamidronate on aggrecanase activity. Direct effect of estradiol or pamidronate on cartilage explants resulted in a decrease only with the high dose of estradiol (87.7±24.9%; p=0.049) and with pamidronate (97.8±8.9%; p=0.049). These results were consistent with data obtained with ARGSV WB.
Conclusion: These ex vivo results suggest that bone soluble factors are capable of modulating the remodeling of articular cartilage and indicate the involvement of catabolic mechanisms. These data provide further evidence of a cross-talk between bone and cartilage and highlights bone as a potential target for osteoarthritis therapy.

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EXPRESSION OF THE GROWTH FACTOR PLEIOTROPHIN AND ITS RECEPTOR PROTEIN TYROSINE PHOSPHATASE BETA/ZETA (RPTPβ/ζ) IN PATIENTS WITH OSTEOARTHRITIS

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Purpose: Pleiotrophin (PTN) is a heparin-binding growth factor expressed in the cartilage in foetal and young age, but its exact role remains unclear as yet. The purpose of this paper is to study the expression of PTN and its receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) in the cartilage and the subchondral bone of patients with osteoarthritis.
Methods: We studied the cartilage and the subchondral bone from 29 patients who had undergone total knee and hip arthroplasty for osteoarthritis, by using Western blot and immunohistochemistry analyses. As controls, we used eight patients operated for fractures of femoral head, who did not present radiological or macroscopical osteoarthritic changes
Results: PTN and RPTPβ/ζ were not detected in the cartilage of normal adults. Their expression and interaction were increased in patients with osteoarthritis (OA) of moderate radiological and histological severity. PTN was detected mainly in the cytoplasm, in clusters of superficial chondrocytes, in necrotic area chondrocytes and in chondrocytes in the tidemark zone. Moreover, it was also expressed in subchondral bone osteocytes, with maximum expression in moderate OA. RPTP β/ζ was also found to be expressed in the subchondral bone in moderate OA, while, as the severity of the disease increased, it was found to be expressed in the osteocytes of the trabecular bone.
Conclusions: The increased expression of PTN and RPTPβ/ζ in the cartilage and subchondral bone of patients with OA renders these molecules potentially interesting candidate targets for developing a therapeutic approach to the disease

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INHIBITION OF PROSTAGLANDIN E2 AND MATRIX METALLOPROTEINASES SYNTHESIS IN INTERLEUKIN-1B-STIMULATED OSTEOBLASTS: A POTENTIAL ROLE OF CHONDROITIN SULFATE ON BONE IN OSTEOARTHRITIS

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Purpose: Osteoarthritis is not only characterized by articular cartilage destruction but also by an abnormal bone remodeling characterized by subchondral bone sclerosis and osteophyte formation A growing number of studies indicate that subchondral bone cells, such as osteoblasts, could partake in the disease process by releasing mediators involved in articular cartilage degradation.
Chondroitin sulfate (CS) is a major component of the extracellular matrix of many connective tissues. This sulfated glycosaminoglycan (GAG), which is referred as a "symptomatic slow-acting drug in OA" (SySADOA), has been shown to have anti-inflammatory and anti-catabolic properties on chondrocytes but little is known about its action on osteoblasts. The objective of this study was, therefore, to determine the effect of CS on inflammatory mediators and proteolytic enzymes induced by interleukin-1β (IL-1β) and related to cartilage catabolism in murine osteoblasts.
Methods: Osteoblasts were obtained by enzymatic digestion of calvaria cortical bone from 5-6-days-old Swiss mice and cultured for 3 weeks as a primary culture. Cells were then stimulated with 1 or 10 ng/ml of IL-1β for 24 hours. CS-treated osteoblasts were incubated with 100 μg/ml of CS during the last week of culture w/o IL-1β. Expressions of cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1 (mPGES-1), 15-hydroxy-prostaglandin dehydrogenase (15-PGDH), matrix metalloproteinase (MMP)-3 and -13 were determined by real-time PCR. PGE_2 and MMP-3 releases were analyzed in the medium by enzyme-linked immunosorbent assay.
Results: As expected, IL-1β (at doses of 1 and 10 ng/ml) increased COX-2, mPGES-1, MMP-3, MMP-13 expression, decreased 15-PGDH expression, and increased PGE2 and MMP-3 release. Interestingly, CS treatment significantly decreased IL-1β-induced expression of COX-2 (by 41% and 65% for stimulation with 1 ng/ml and 10 ng/ml of IL-1β, respectively), mPGES-1 (by 36% and 55%), MMP-3 (by 25% and 44%) and MMP-13 (by 19% and 49%). Accordingly, PGE_2 and MMP-3 releases were decreased by 57% and 38% respectively when cells were stimulated with 1 ng/ml of IL-1β, and by 84% and 50% when cells were stimulated with 10 ng/ml of IL-1β.
Conclusions: Chondroitin sulfate represses the osteoblastic synthesis of inflammatory mediators and proteolytic enzymes involved in cartilage degradation. These data indicate that the beneficial effects of CS in OA could not only be due to its action on cartilage but also to its beneficial effects on subchondral bone.

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SECRETED FACTORS FROM MATURE OSTEOCLASTS COULD CONTRIBUTE TO THE PATHOLOGY OF OSTEOARTHRITIS AUGMENTING BONE FORMATION AND LOWERING CARTILAGE FORMATION

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Purpose: Osteoarthritis is a degenerative disease characterized by opposing processes within cartilage and the subchondral bone: cartilage degradation and osteosclerosis. Osteoclasts themselves are known to have importance for the coupling between bone resorption and bone formation, as these cells secrete factors which induce bone formation. However, it is currently unknown, whether osteoclasts have direct effects on cartilage turnover. The aim of this study was to further evaluate the effect of osteoclast secreted factors on both bone formation and cartilage turnover.
Methods: Human monocytes were isolated from blood and differentiated into mature osteoclasts using M-CSF and RANKL. Conditioned medium (CM) and corresponding non-conditioned medium (non-CM) were collected during culture. The pre-osteoblastic cell line 2T3 and bovine cartilage explants were subsequently treated with 50% of CM or non-CM in addition to relevant controls. Bone formation was assessed by Alizarin Red staining/dye extraction and cartilage formation was measured by PIINP ELISA. Cartilage degradation was evaluated by ELISA measurements of both MMP mediated collagen type II (COL II) degradation and aggrecan cleavage, in addition to histological evaluations of Safranin-O stained explants following treatments.
Results: Treatment of 2T3 cells with CM from mature osteoclasts (day 7-10 of culture), strongly induced bone formation by 200%, whereas CM from monocytes and aged osteoclasts had no bone anabolic effect. CM from mature osteoclasts, with bone anabolic effect, was furthermore tested in cartilage explant cultures, where it was demonstrated that cartilage formation was significantly decreased by 70%. In addition, degradation of aggrecans was increased, measured by both ELISA and histological evaluations. In contrast, MMP mediated degradation of COL II was unchanged.
Conclusions: Osteoclasts with a specific maturity have the ability to stimulate bone formation by secretion of bone anabolic signals. However, the same CM had opposite effects on cartilage, namely reduced formation and accelerated aggrecan degradation. All together, these data point in the direction of osteoclasts having important roles, for both bone and cartilage turnover. These observations could be of specific relevance for osteoarthritis pathology, in which cartilage catabolic and bone anabolic processes are taking place at the same time.